Sex differences in heart failure

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Chapter 4

Neurohormonal and clinical sex differences in heart failure.

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Abstract

Aims

Despite disparities in pathophysiology and disease manifestation between male and female patients with heart failure, studies focusing on sex differences in biomarkers are scarce. The purpose of this study was to assess sex-specific variation in clinical characteristics and biomarker levels to gain more understanding of the potential pathophysiological mechanisms underlying sex differences in heart failure.

Methods and results

Baseline demographic and clinical characteristics, multiple biomarkers, and outcomes were compared between men and women in 567 patients. The mean age of the study group was 71 ± 11 years and 38% were female. Women were older, had a higher body mass index and left ventricular ejection fraction, more hypertension, and received more diuretic and antidepressant therapy, but less ACE-inhibitor therapy compared with men. After 3 years, all-cause mortality was lower in women than men (37.0 vs. 43.9%, multivariable hazard ratio = 0.64; 95% confidence interval 0.45–0.92, P = 0.016). Levels of biomarkers related to inflammation [C-reactive protein, pentraxin 3, growth differentiation factor 15 (GDF-15), and interleukin 6] and extracellular matrix remodelling (syndecan-1 and periostin) were significantly lower in women compared with men. N-terminal pro-brain natriuretic peptide, TNF-αR1a, and GDF-15 showed the strongest interaction between sex and mortality.

Conclusion

Female heart failure patients have a distinct clinical presentation and better outcomes compared with male patients. The lower mortality was independent of differences in clinical characteristics, but differential sex associations between several biomarkers and mortality might partly explain the survival difference.

Introduction

Heart failure (HF) is a clinical syndrome that affects both men and women. Although the total number of men and women living with heart failure is similar,[1] female patients are underrepresented in clinical studies in heart failure.[2,3] Therefore, evidence relating to pathophysiology, aetiology, clinical presentation, treatment, and outcome is predominantly based on data from male patients.[4] A few major pharmacological and device trials in heart failure patients have performed sex-specific analyses. In contrast to patients with cardiovascular disease, these trials consistently reported an independent survival benefit for women.[5–8] Sex-specific analysis of the Candesartan in Heart failure assessment of Mortality and Morbidity (CHARM)[6] trial showed that differential survival is independent of age, left ventricular ejection fraction (LVEF) and the cause of heart failure. Sex-dependent differences in survival were also recently demonstrated in the Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy (MADIT-CRT),[8] with women more frequently showing reverse cardiac remodelling. However, the basic biological mechanisms related to the sex difference with regard to outcomes have not been properly addressed, despite known pathophysiological disparities involving inflammation and remodelling.[9] We hypothesized that sex-specific differences in mortality are associated with disparities in biomarkers indicative of inflammation and remodelling. We performed sex-specific analyses on the variation in basic demographic and clinical characteristics, clinical outcomes, and levels of different biomarkers of inflammation, oxidative stress, remodelling, and cardiomyocyte stretch in a large number of heart failure patients.

Methods

Study design and population

The Coordinating study evaluating Outcomes of Advising and Counseling in Heart failure (COACH) data set was used. COACH was a multicentre, randomized, controlled, nurse-led disease

management intervention trial testing whether follow-up by a cardiologist or basic or intensive additional support by a heart failure nurse improve outcomes in patients hospitalized with heart failure. No reduction of the combined endpoints of death and heart failure-related hospitalizations were seen with any intervention compared with the standard follow-up. Rationale, design, and detailed results have previously been reported elsewhere.[10–14] The original COACH study included 1023 patients shortly before discharge following a heart failure hospitalization. Patients with the full continuum of LVEF were enrolled. This study refers to the subset of 567 patients from the COACH cohort, in whom samples for biomarker analysis were obtained.

Study measures and laboratory tests

The primary outcome measure for the present analyses was all-cause mortality within ~3 years (up to 1124 days). Secondary outcome variables were time to death or heart failure hospitalization and the number of days lost to death or hospitalization at 18 months. All other data were obtained at index hospitalization. Heart Failure with preserved Ejection Fraction was defined by LVEF ≥50%. The CES-D score[15,16] was used for the assessment of depression with a score ≥16 indicating depressive symptoms; the quality of life was quantified using the Minnesota Living with Heart Failure questionnaire (MLHFQ).[17] Post hoc analyses of biomarkers encompassed the markers displayed in Table 2. Biomarker analysis was performed using the following commercial assays: C-reactive protein, pentraxin 3 (PTX3), growth differentiation factor 15 (GDF-15), myeloperoxidase (MPO), galectin 3, syndecan-1, periostin, ST-2, tumour necrosis factor alpha (TNF-α), TNF-αR1a, osteopontin, RAGE, angiogenin, endothelial cell-selective adhesion molecule (ESAM), cystatin C, and neutrophil gelatinase-associated lipocalin (NGAL) were measured by Alere San Diego, Inc., San Diego, CA, USA, using competitive enzyme-linked immunosorbent assays (ELISAs) on a Luminex® platform. Transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) were analysed using a quantitative multiplexed sandwich ELISA system, SearchLight® proteome

arrays, Aushon BioSystems, Billerica, MA, USA. N-terminal pro-brain natriuretic peptide (NTpro-BNP) was measured using the Elecsys proBNP ELISA by Roche Diagnostics, Mannheim, Germany. Erythropoietin alpha (EPOa) was measured using the IMMULITE® EPO ELISA by Diagnostic Products Corporation, Los Angeles, CA, USA. Estimated glomerular filtration rate (eGFR) was based on the simplified Modification of Diet in Renal Disease (MDRD) formula. Anaemia was diagnosed using the World Health Organization (WHO) definition with a haemoglobin threshold of 13.0 g/dL in men and 12.0 g/dL in women.

Table 1

Baseline characteristics

	Total cohort (*n* = 567)	**Male (*n* = 351)**	**Female (*n* = 216)**	***P*-value**
Demographics and HF characteristics				
Age, mean ± SD, years	71.0 ± 11.0	69.9 ± 10.6	72.7 ± 11.4	0.004
Left ventricular EF, mean ± SD, %	32.5 ± 14.0	31.0 ± 13.0	34.9 ± 15.3	0.004
Preserved EF, *n* (%)	70 (15.2)	34 (11.8)	36 (20.9)	0.008
Ischaemic aetiology, *n* (%)	232 (40.9)	168 (47.9)	64 (29.6)	0.000
Duration of HF, median (IQR)	112 (22–1401)	123 (23–1336)	84 (21–1447)	0.770
Anaemia, *n* (%)	118 (37.8)	50 (26.2)	68 (56.2)	0.000
NYHA class (II/III/IV), %	5.0/54.7/40.3	6.0/53.7/40.3	3.3/56.3/40.4	0.345
MLHF Questionnaire, median (IQR)	45.0 (28.0–60.0)	45.0 (28.0–61.0)	46.0 (29.0–59.0)	0.936
Clinical signs				
Weight, mean ± SD, kg	77.4 ± 16.8	80.5 ± 16.1	72.2 ± 16.7	0.000
Body mass index, mean ± SD, kg/m^2	27.1 ± 5.5	26.7 ± 4.8	27.9 ± 6.6	0.020
Systolic blood pressure, mean ± SD, mmHg	118.2 ± 21.2	116.7 ± 20.6	120.6 ± 21.9	0.034
Ankle oedema, *n* (%)	359 (64.5)	214 (62.2)	145 (68.1)	0.160
Comorbidities [*n* (%)]				
Hypertension	240 (42.3)	135 (38.5)	105 (48.6)	0.018
Diabetes	173 (30.5)	96 (27.4)	77 (35.7)	0.037
Atrial fibrillation or flutter	261 (46.0)	166 (47.3)	95 (44.0)	0.442
Chronic obstructive pulmonary disease	159 (28.0)	111 (31.6)	48 (22.2)	0.016
Depression (CES-D score ≥6)	208 (39.3)	123 (37.1)	85 (42.9)	0.189
Medication [*n* (%)]				
ACE-inhibitor	286 (50.4)	189 (53.9)	97 (44.9)	0.039
Angiotensin receptor blocker	71 (12.5)	45 (12.8)	26 (12.0)	0.784
Beta-blocker	250 (44.1)	161 (45.9)	89 (41.2)	0.277
Spironolactone	166 (29.3)	92 (26.2)	74 (34.3)	0.041
Diuretic	438 (77.3)	258 (73.5)	180 (83.3)	0.007
Digoxin	146 (25.8)	90 (25.6)	56 (25.9)	0.940
Antidepressants	34 (6.0)	12 (3.4)	22 (10.2)	0.001

EF, ejection fraction; *HF*, heart failure; *NYHA*, New York Heart Association; *MLHF*, Minnesota Living with Heart Failure; *CES-D*, Center for Epidemiologic Studies Depression; *ACE*, angiotensin-converting

Table 2

Sex-specific biomarker levels

	Total cohort (*n* = 567)	Male (*n* = 351)	Female (*n* = 216)	*P*-value
Inflammation				
C-reactive protein, μg/mL	11.4 (4.8–33.0)	13.0 (5.5–33.0)	9.0 (4.2–28.9)	0.018
PTX 3, ng/mL	3.7 (2.5–5.6)	3.9 (2.7–5.8)	3.3 (2.2–5.0)	0.002
GDF-15, ng/mL	2.8 (1.9–4.3)	3.1 (2.2–4.7)	2.4 (1.7–3.8)	0.000
Osteopontin, ng/mL	159.2 (109.0–223.1)	165.7 (111.4–232.8)	147.2 (100.9–209.3)	0.083
RAGE, ng/mL	2.9 (1.9–4.6)	3.0 (1.9–4.7)	2.7 (1.9–4.2)	0.165
Interleukin 6, ng/mL	12.0 (6.8–24.3)	13.1 (7.9–28.4)	10.9 (5.9–18.4)	<0.001
TNF-α, pg/mL	45.8 (4.7–121.3)	47.3 (4.7–146.4)	43.7 (4.8–85.0)	0.230
TNF-αR1a, ng/mL	3.1 (2.2–4.6)	3.1 (2.2–4.7)	2.9 (2.2–4.4)	0.500
Oxidative stress				
MPO, ng/mL	20.1 (15.6–28.1)	20.4 (15.7–28.4)	19.1 (15.3–26.5)	0.115
Remodelling				
Syndecan-1, ng/mL	20.8 (15.4–28.5)	20.8 (15.4–28.5)	17.7 (12.2–26.1)	0.004
Periostin, ng/mL	4.7 (3.4–6.6)	5.0 (3.5–6.6)	4.4 (3.1–6.3)	0.023
Galectin 3, ng/mL	25.6 (21.1–32.1)	26.2 (21.5–32.5)	24.9 (20.2–31.2)	0.057
TGF-β, ng/mL	51 (35–75)	48 (34–72)	53 (36–82)	0.043
Cardiomyocyte stretch				
NTpro-BNP, pg/mL	2532 (1309–5721)	2677 (1407–6340)	2344 (1197–5047)	0.978
ST-2, ng/mL	2.5 (1.4–5.4)	2.6 (1.5–5.4)	2.2 (1.2–5.5)	0.069
Angiogenesis				
VEGF, ng/mL	63.0 (31.4–143.8)	58.7 (27.3–118.0)	73.1 (36.8–189.4)	0.003
Angiogenin, μg/mL	5.1 (3.6–7.5)	5.0 (3.6–7.4)	5.3 (3.5–8.0)	0.465
Arteriosclerosis				
ESAM, ng/mL	53.0 (44.5–64.3)	54.1 (45.5–65.1)	51.3 (43.0–62.1)	0.038
Renal function				
eGFR, mL/min/1.73m^2	53.9 ± 20.2	55.8 ± 19.9	50.9 ± 20.2	0.006
Cystatin C, μg/mL	11.1 (7.6–16.2)	11.1 (7.7–16.9)	11.1 (7.6–15.7)	0.774
NGAL, ng/mL	84.6 (60.4–123.3)	85.8 (61.3–135.9)	83.8 (58.8–116.1)	0.127
Anaemia				
Hb, g/dL	13.1 ± 2.0	13.4 ± 2.1	12.6 ± 1.8	<0.001
EPOa, IU/L	9.6 (5.2–16.0)	9.7 (5.1–16.5)	9.5 (5.2–15.0)	0.569

PTX3, pentraxin 3; *GDF-15*, growth differentiation factor 15; *RAGE*, receptor for advanced glycation end products; *TNF-α*, tumour necrosis factor alpha; *TNF-αR1a*, tumour necrosis factor alpha receptor 1a; *MPO*, myeloperoxidase; *TGF-β*, transforming growth factor-beta; *NTpro-BNP*, N-terminal pro-brain natriuretic peptide; *ST-2*, suppression of tumourigenicity 2; *VEGF*, vascular endothelial growth factor; *EPOa*, erythropoietin alpha; *ESAM*, endothelial cell-selective adhesion molecule; *NGAL*, neutrophil gelatinase-associated lipocalin.

Statistical analyses

Continuous variables are presented as mean ± SD or median with inter-quartile range, where appropriate. Categorical variables are presented as counts and percentages. Comparisons of

continuous variables were performed using either Student's t test or the Mann–Whitney test, as appropriate. The $\chi 2$ test was used to test for categorical variables.

Hazard ratios (HRs) were calculated using univariable and multivariable Cox proportional hazards regression. The proportionality assumption for the Cox regression analysis was evaluated on the basis of Schoenfeld residuals.

Biomarkers were used on a continuous scale for baseline sex-comparison and with log-transformation in Cox proportional hazards models.

First, univariable Cox proportional hazards regression analyses of the sex-specific outcome were performed using baseline characteristics and previously established cofounders of the COACH Risk engine.[18]

Secondly, multivariable Cox proportional hazards regression was performed, adjusting for variables, which showed univariable association with 3-year mortality at $P < 0.1$ in this cohort. The variables entered to the multivariable model comprised: age, ischaemic aetiology (i.e. previous myocardial infarction), duration of HF, MLHFQ score, weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, ankle oedema, diabetes, atrial fibrillation, ACE-inhibitor therapy, beta-blocker therapy, aldosterone antagonist therapy, diuretic therapy, digoxin therapy, stroke, peripheral vascular disease, previous heart failure hospitalization, serum sodium.

Thirdly, comprehensive multivariable modelling was performed, separately adding the respective biomarkers to the model to detect the most relevant change in point estimates for relative hazard ratios. Furthermore, we studied the interaction of the individual biomarkers with the sex-effect on mortality. Statistical analyses were performed using the STATA (version 11.0, STATA Corp, College Station, TX, USA) and R (version 2.15.1, R Foundation for Statistical Computing, Vienna, Austria) software. A two-sided P-value < 0.05 was considered statistically significant.

Results

Baseline demographic and clinical characteristics Of the 567 patients of COACH included in this analysis 216 (38%) were female (Table 1). On average, women were 2.7 years older, showed 3.9% higher absolute LVEF and a greater proportion of preserved LVEF than men. Ischaemic aetiology of heart failure was significantly less prevalent in women. Anaemia was more than twice as common in women when compared with men. The duration of heart failure, NYHA functional class and the Minnesota Living with Heart Failure Questionnaire (MLHFQ) score did not differ between sexes.

Women had 8.3 kg lower average body weight, but 1.1 kg/m2 higher BMI. Systolic blood pressure was higher by 3.9 mmHg in women compared with men. Hypertension and diabetes were more common in women, while chronic obstructive pulmonary disease was more prevalent in men. No difference between male and female patients was found regarding atrial fibrillation or flutter; neither for signs of depression. However, women used more antidepressants compared with men. Women also received less ACE-inhibitor therapy, but more diuretics and spironolactone compared with men.

Biomarker levels

Table 2 provides an overview of the biomarker levels in male and female patients. Women had consistently lower values than men for inflammatory markers C-reactive protein, PTX3, GDF-15, and Interleukin 6, while no statistical difference was detectable for osteopontin, TNF-α and TNF-αR1a, and MPO, a marker of oxidative stress. In addition, lower levels of the remodelling markers syndecan-1 and periostin were found in women, while lower galectin-3 levels were not significant. Transforming growth factor-beta was significantly higher in women compared with men. Levels of the myocardial stretch markers NTpro-BNP and ST-2 were not significantly different between sexes. The angiogenesis marker VEGF was significantly higher in women, while there was no sex difference for angiogenin. Endothelial cell-selective adhesion molecule, a marker of arteriosclerosis, was significantly lower in female compared with male patients. No sex differences in the levels of the

biomarkers of renal function cystatin C and NGAL were found, whereas eGFR was lower by 4.9 mL/min/1.73 m2 in women compared with men. Haemoglobin levels were 0.8 g/dL lower in women, whereas no different levels of the erythropoiesis marker EPO could be detected between both sexes.

Outcomes

Mortality

The estimated 3-year event rates are 44% (39–49%) for males and 37% (31–44%) for females, respectively. Table 3 shows the age-adjusted and multivariable association of sex with mortality. Female heart failure patients had lower age-adjusted 3-year all-cause mortality compared with male patients (HR = 0.71; 95% CI: 0.54–0.93, P = 0.014). Figure 1 shows the sex-specific Kaplan–Meier curves for 3-year all-cause mortality. In a multivariable model, we adjusted for the clinical risk markers for mortality in this patient cohort. Even after full adjustment, female sex was associated with a 36% lower mortality risk (HR = 0.64; 95% CI 0.45–0.92, P = 0.016). The proportionality assumption held ($\chi 2 = 16.92$; P = 0.716).

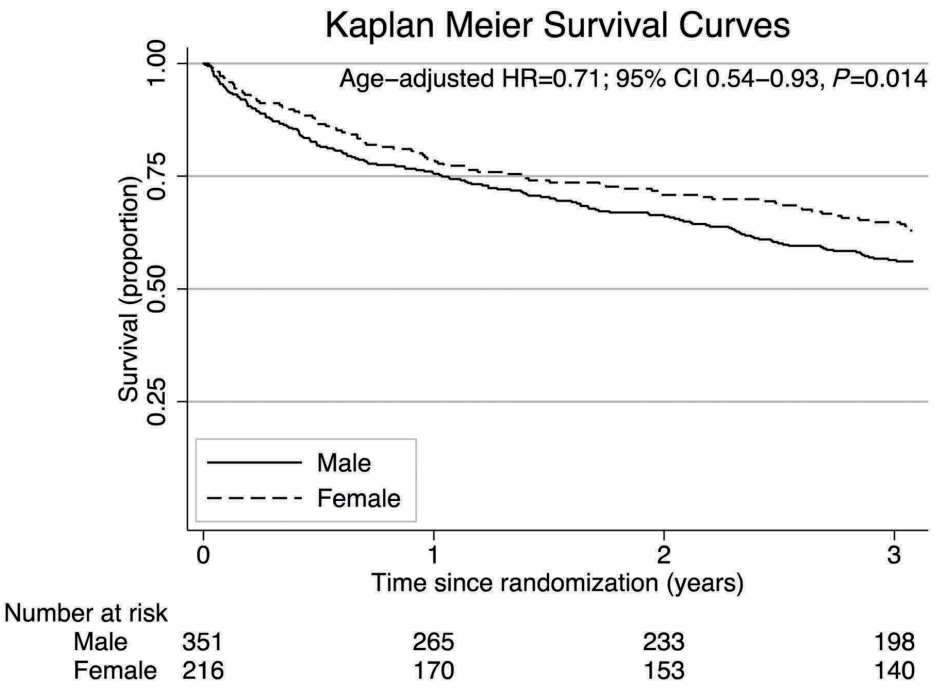
Table 3

Sex-specific outcome analyses

Age-adjusted and multivariable model		
Variable	**Sex**	
n = 567	**Adjusted HR (95% CI)**	**Z (P)**
Age adjusted	0.71 (0.54–0.93)	−2.45 (0.014)
Multivariable	0.64 (0.45–0.92)	−2.41 (0.016)

Figure 1

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality.



Biomarkers

Table 4 shows the association between the individual biomarkers, sex, and mortality and also the P-values for interaction of individual biomarkers with sex. Concerning the association between female sex and mortality, the change in point estimate for the hazard ratio was most pronounced when adding NTpro-BNP [from 0.64 (0.45–0.92) to 0.79 (0.54–1.14)] and GDF-15 [from 0.64 (0.45–0.92) to 0.73 (0.50–1.05)]. In addition to NTpro-BNP and GDF-15, we found that TNF-αR1a, MPO, syndecan, galectin 3, and ESAM had a different prognostic value in male vs. female patients. Kaplan–Meier survival curves for all biomarkers with significant interaction between sex and outcome are shown in Figures 2–8.

Table 4

Sex-specific biomarker—outcome and interaction analyses

Covariate	Point estimate for sex (multivariable model + individual biomarker)		Interaction (multivariable model + interaction term)
	Adjusted HR (95% CI)	Z (*P*)	*P*-value
Inflammation			
C-reactive protein ($n = 567$)	0.66 (0.46–0.94)	−2.30 (0.022)	0.217
PTX ($n = 567$)	0.68 (0.47–0.97)	−2.10 (0.035)	0.105
GDF-15 ($n = 567$)	0.73 (0.50–1.05)	−1.72 (0.086)	0.072
Osteopontin ($n = 567$)	0.65 (0.46–0.94)	−2.30 (0.021)	0.548
RAGE ($n = 567$)	0.64 (0.44–0.91)	−2.45 (0.014)	0.214
Interleukin 6 ($n = 526$)	0.71 (0.48–1.05)	−1.71 (0.087)	0.272
TNF-α ($n = 464$)	0.58 (0.38–0.87)	−2.65 (0.008)	0.567
TNF-αR1a ($n = 567$)	0.66 (0.46–0.95)	−2.24 (0.025)	0.057
Oxidative stress			
MPO ($n = 567$)	0.64 (0.47–0.92)	−2.43 (0.015)	0.078
Remodelling			
Syndecan ($n = 567$)	0.66 (0.46–0.95)	−2.23 (0.026)	0.093
Periostin ($n = 567$)	0.65 (0.45–0.93)	−2.38 (0.017)	0.333
Galectin 3 ($n = 567$)	0.63 (0.44–0.91)	−2.47 (0.014)	0.084
TGF-β ($n = 547$)	0.62 (0.43–0.90)	−2.52 (0.012)	0.277
Cardiomyocyte stretch			
NTpro-BNP ($n = 538$)	0.79 (0.54–1.14)	−1.25 (0.212)	0.039
ST-2 ($n = 567$)	0.65 (0.45–0.93)	−2.36 (0.018)	0.624
Angiogenesis			
VEGF ($n = 515$)	0.65 (0.45–0.92)	−2.39 (0.017)	0.283
Angiogenin ($n = 567$)	0.65 (0.45–0.94)	−2.31 (0.021)	0.318
Arteriosclerosis			
ESAM ($n = 567$)	0.65 (0.45–0.93)	−2.37 (0.018)	0.082
Renal function			
eGFR ($n = 557$)	0.60 (0.41–0.85)	−2.81 (0.005)	0.135
Cystatin C ($n = 567$)	0.65 (0.45–0.93)	−2.36 (0.019)	0.723
NGAL ($n = 562$)	0.69 (0.48–0.99)	−2.01 (0.044)	0.141
Anaemia			
Hb ($n = 312$)	0.52 (0.32–0.85)	−2.61 (0.009)	0.588
EPOa ($n = 565$)	0.64 (0.45–0.92)	−2.41 (0.016)	0.239

The change in point estimates for relative hazard ratios of the sex- and biomarker-variables if the respective biomarkers are added separately to a model adjusting for variables which showed univariate association with 3-year mortality at $P < 0.1$: age, ischaemic aetiology (i.e. previous myocardial infarction), duration of HF, MLwHF score, weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, ankle oedema, diabetes, atrial fibrillation, ACE-inhibitor therapy, beta-blocker therapy, aldosterone antagonist therapy, diuretic therapy, digoxin therapy, stroke, peripheral vascular disease, previous heart failure hospitalization, and serum sodium.
PTX3, pentraxin 3; *GDF-15*, growth differentiation factor 15; *RAGE*, receptor for advanced glycation end products; *TNF-α*, tumour necrosis factor alpha; *TNF-αR1a*, tumour necrosis factor alpha receptor 1a; *MPO*, myeloperoxidase; *TGF-β*, transforming growth factor-beta; *NTpro-BNP*, N-terminal pro-brain natriuretic peptide; *ST-2*, suppression of tumourigenicity 2; *VEGF*, vascular endothelial growth factor; *ESAM*, endothelial cell-selective adhesion molecule; *eGFR*, estimated glomerular filtration rate; *NGAL*, neutrophil gelatinase-associated lipocalin; *Hb*, haemoglobin; *EPOa*, erythropoietin alpha.

Figure 2

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on growth differentiation factor-15 tertiles for men (A) and women (B).

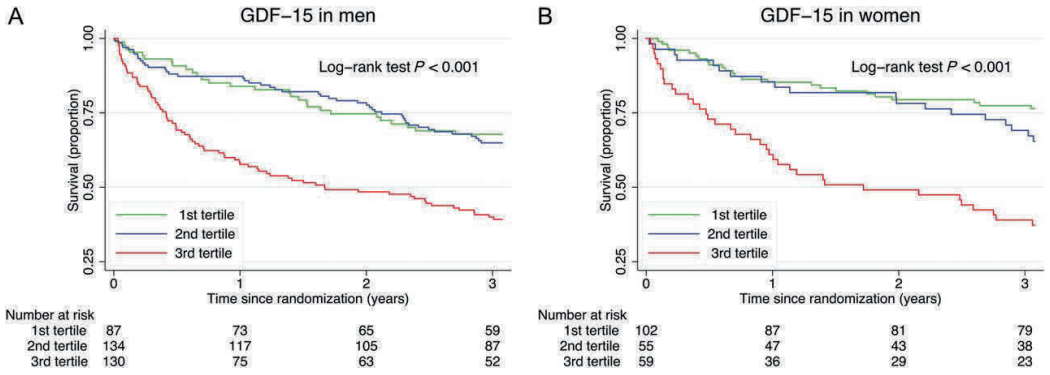


Figure 3

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on tumour necrosis factor-αR1a tertiles for men (A) and women (B).

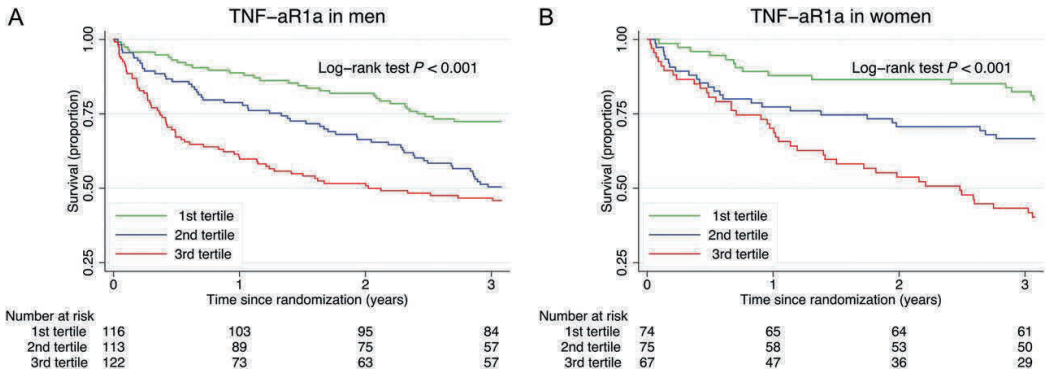


Figure 4

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on myeloperoxidase tertiles for men (A) and women (B).

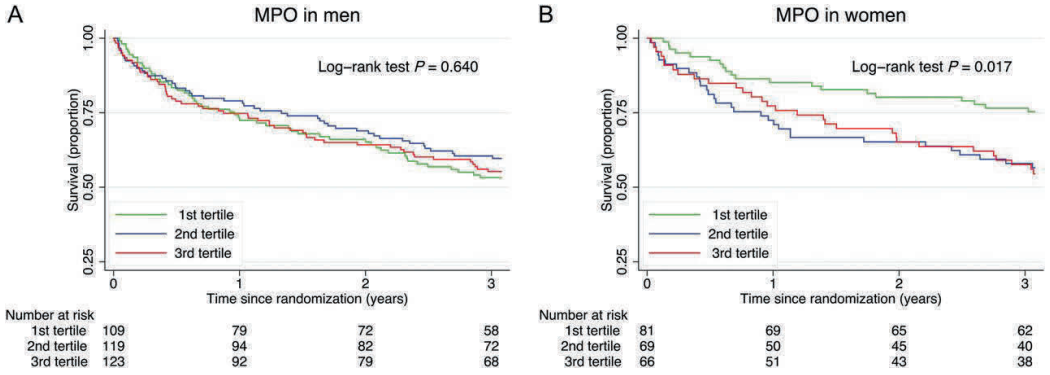


Figure 5

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on syndecan tertiles for men (A) and women (B).

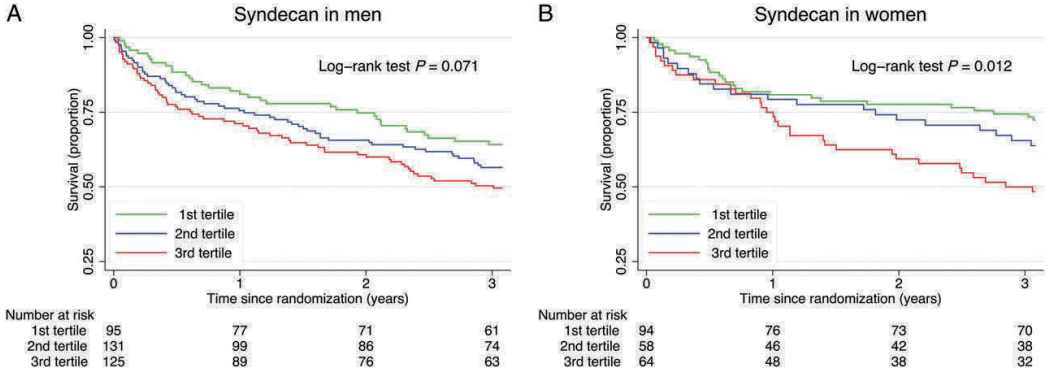


Figure 6

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on galectin 3 tertiles for men (A) and women (B).

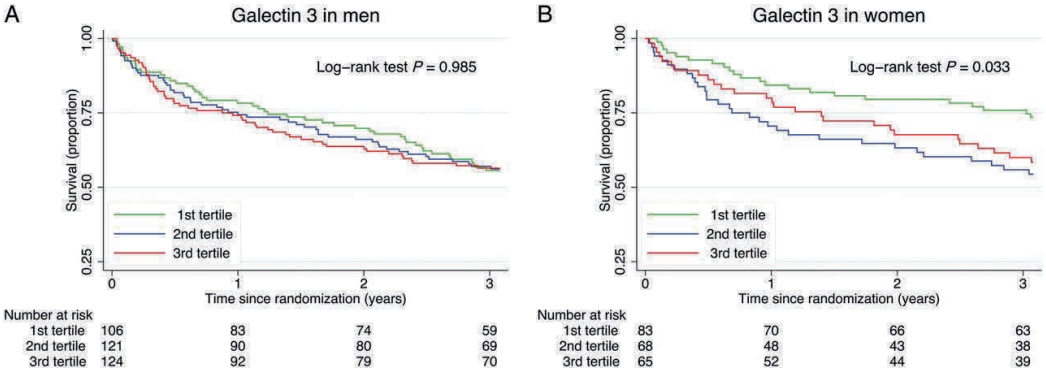


Figure 7

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on N-terminal pro-brain natriuretic peptide tertiles for men (A) and women (B).

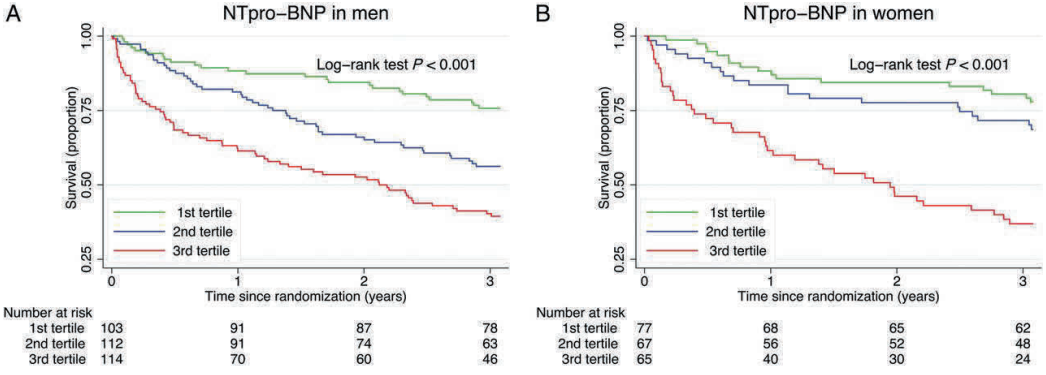
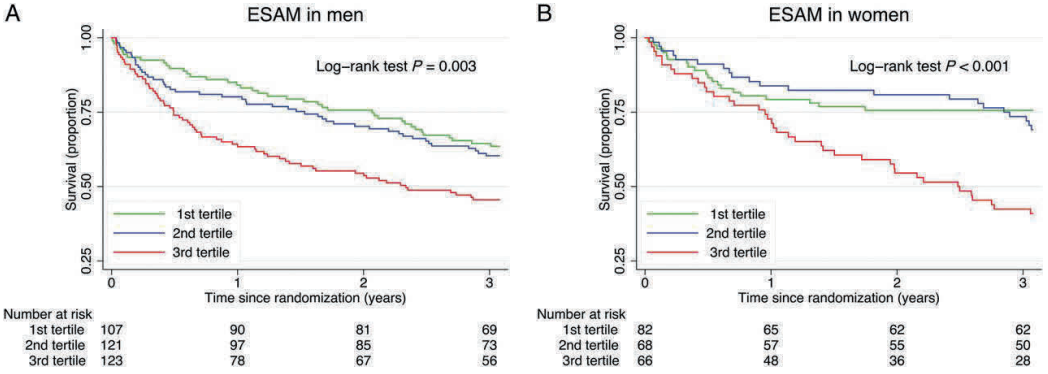


Figure 8

Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on endothelial cell-selective adhesion molecule tertiles for men (A) and women (B).



Discussion

This is the first study to report on biomarker-related differences between male and female heart failure patients. We confirmed that female heart failure patients have a better prognosis compared with male patients, which could not be explained by the difference in clinical characteristics. Interestingly, several biomarkers were lower in women, and in addition to NTpro-BNP, GDF-15, TNF-αR1a, MPO, syndecan, galectin 3, and ESAM had a sex-dependent prognostic value.

Sex-related clinical characteristics

Female patients in COACH showed the typical female clinical presentation pattern of heart failure, which has been characterized in many other studies[5–7,19] and registries.[20,21] Female heart failure patients are generally older, have more preserved LVEF, suffer less frequently from ischaemic cardiomyopathy, and show more hypertension and signs of congestion compared with male patients. These classic sex-specific manifestations could be interpreted as reflections of the underlying sex

disparities in pathophysiology and natural development of heart failure over time. However, these differences alone do not explain the survival benefit in women.

Survival differences

The survival benefit for women in the present study is consistent with the results of other studies: O'Meara et al.6 reported an independent survival benefit for women in the CHARM trial, accounting for LVEF and the cause of heart failure [adjusted hazard ratio (HR), 0.77; 95% CI: 0.69 to 0.86; P < 0.001]. Notably, this large cohort comprised patients with both reduced and preserved EF. Alla et al.[22] published the sex-specific findings of the Digitalis Investigation Group (DIG) trial showing comparable results, with independent survival benefit for women irrespective of LVEF, cause of heart failure or duration of heart failure. Similarly, population-based studies consistently report lower mortality for female heart failure patients,[23,24] precluding trial-specific selection bias as explanation for the survival benefit. A recent large individual patient data meta-analysis powerfully supports this finding.[25] However, none of the studies performed to date has adequately clarified a biological background for the survival benefit for women with heart failure.

Biomarkers

Overall, lower baseline levels of biomarkers indicative of inflammation and remodelling suggest less biological activity in the respective pathophysiological pathways in women compared with men. This may imply a different biological disease expression, but could also reflect natural biological variation between sexes. However, in healthy populations, women show higher basic levels of C-reactive protein,[26] PTX3,[27] RAGE,[28] galectin 3,[29] and NTpro-BNP[30]. Other studies report that GDF-15,[31] VEGF, NGAL,[32] and EPOa[33] were similar in men and women. Lower normal levels of TNF-α,[34] TGF-β,[35] ESAM,[36] GFR,[37] cystatin C, and haemoglobin[38] have been reported in women. Sex-specific population-based data are scarce for the remaining markers. Reference levels from cohorts of

healthy volunteers of each sex can be found in Table SI. This suggests that women hospitalized for heart failure have a distinct biological disease expression compared with men.

Table SI Reference levels from cohorts of healthy volunteers of each sex

	Male	Female	Reference	P Value
Inflammation				
CRP, µg/mL	1.30 (2.2) n = 1253	2.47 (4.1) n = 1349	Lakoski SG et al.[1]	<.0001
PTX 3, ng/mL	1.87 (1.81-1.94) n = 818	2.12 (2.05-2.19) n = 931	Yamasaki K et al.[2]	0.0001
GDF-15, ng/L	749 (588–957) n = 288	780 (610–967) n = 141	Kempf T et al.[3]	0.507
Osteopontin, ng/mL	382 (257-540) n = 43		Rosenberg M et al.[4]	
RAGE, pg/mL	1414 ± 649 n = 121	1744 ± 660 n = 55	Norata GD et al.[5]	<0.05
TNF-α, pg/mL	3.05 (1.90–4.65) n = 2884	2.75 (1.73–4.42) n = 3201	Marques-Vidal P et al.[6]	<0.001
TNF-αR1a, pg/mL	1506 ± 541 n = 529	1267 ± 354 n = 469	Pai JK et al.[7]	
IL-6, pg/mL	1.53 (0.98–2.88) n = 529	1.65 (1.15–2.65) n = 469	Pai JK et al.[7]	
Oxidative Stress				
MPO, ng/mL	657 (464-985) n = 1411	607 (446-894) n = 826	Meuwese MC et al.[8]	
Remodeling				
Syndecan-1, ng/mL	19.86 (14.49-33.14) ng/ml n = 32		Jilani I et al.[9]	
Periostin, ng/mL	21.0 ± 7.3 n = 120		Ben QW et al.[10]	
Galectin 3, ng/mL	10.7 (8.9-12.7) n = 4140	11 (9.1-13.4) n = 4182	PREVEND study *unpublished*	<0.001
TGF-β, ng/mL	37.6 ± 0.12 n = 4888	35.1 ± 0.12 n = 4254	Lin Y et al.[11]	<0.001
Cardiomyocyte Stretch				
NTpro-BNP, pg/mL	24.3 (10.1-54.5) n = 4159	50.5 (28.2-87.0) n = 4201	PREVEND study *unpublished*	<0.001
ST-2, ng/mL	0.14 (0.13-0.17) n = 9		Weinberg EO et al.[12]	
Angiogenesis				
VEGF, ng/mL	22,4 (14,8-34,2) n = 270	22,2 (15,0-32,7) n = 113	PREVEND study *unpublished*	0.6239
Angiogenin, ng/mL	150.2 (104.8–202.8) n = 76		Tello-Montoliu A et al.[13]	
Arteriosclerosis				
ESAM, ng/mL	35.2 (27.5-44.5) n = 1418	34.0 (26.8-42.3) n= 1804	Rohatgi A et al.[14]	0.006
Renal function				
GFR, ml/min/1.73m^2	71 ± 12 n = 102	66 ± 11 n = 95	Wetzels JFM et al.[15]	
Cystatin C, µg/mL	0.81 (0.72-0.91) n = 4031	0.74 (0.66-0.84) n = 4098	PREVEND study *unpublished*	<0.001
NGAL, ng/mL	86.3 ± 43.0 n= 53	88.9 ± 38.2.0 n= 83	Stejskal et al.[16]	n.s.
Anaemia				
Haemoglobin, g/dl	15. 3 ± 1.2 n = 1209	13.3 ± 1.2 n= 1286	Lacher DA et al.[16]	<0.001
EPOa, IU/L	9.3 (6.7-13.5) n = 245	8.9 (6.7-11.9) n = 91	Mercadal L et al.[17]	0.2

Supplementary Table SI - References

1. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB,Jr, Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am Heart J 2006;152:593-598.
2. Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K. Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. Clin Chem Lab Med 2009;47:471-477.
3. Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. J Am Coll Cardiol 2007;50:1054-1060.
4. Rosenberg M, Zugck C, Nelles M, Juenger C, Frank D, Remppis A, Giannitsis E, Katus HA, Frey N. Osteopontin, a new prognostic biomarker in patients with chronic heart failure. Circ Heart Fail 2008;1:43-49.
5. Norata GD, Garlaschelli K, Grigore L, Tibolla G, Raselli S, Redaelli L, Buccianti G, Catapano AL. Circulating soluble receptor for advanced glycation end products is inversely associated with body mass index and waist/hip ratio in the general population. Nutr Metab Cardiovasc Dis 2009;19:129-134.
6. Marques-Vidal P, Bochud M, Bastardot F, Luscher T, Ferrero F, Gaspoz JM, Paccaud F, Urwyler A, von Kanel R, Hock C, Waeber G, Preisig M, Vollenweider P. Levels and determinants of inflammatory biomarkers in a Swiss population-based sample (CoLaus study). PLoS One 2011;6:e21002.
7. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 2004;351:2599-2610.
8. Meuwese MC, Stroes ES, Hazen SL, van Miert JN, Kuivenhoven JA, Schaub RG, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, Boekholdt SM. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. J Am Coll Cardiol 2007;50:159-165.
9. Jilani I, Wei C, Bekele BN, Zhang ZJ, Keating M, Wierda W, Ferrajoli A, Estrov Z, Kantarjian H, O'Brien SM, Giles FJ, Albitar M. Soluble syndecan-1 (sCD138) as a prognostic factor independent of mutation status in patients with chronic lymphocytic leukemia. Int J Lab Hematol 2009;31:97-105.
10. Ben QW, Zhao Z, Ge SF, Zhou J, Yuan F, Yuan YZ. Circulating levels of periostin may help identify patients with more aggressive colorectal cancer. Int J Oncol 2009;34:821-828.
11. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. Circulation 2003;107:721-726.
12. Tello-Montoliu A, Marin F, Patel J, Roldan V, Mainar L, Vicente V, Sogorb F, Lip GY. Plasma angiogenin levels in acute coronary syndromes: implications for prognosis. Eur Heart J 2007;28:3006-3011.
13. Rohatgi A, Patel P, Das SR, Ayers CR, Khera A, Martinez-Rumayor A, Berry JD, McGuire DK, de Lemos JA. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. Clin Chem 2012;58:172-182.
14. Wetzels JF, Kiemeney LA, Swinkels DW, Willems HL, den Heijer M. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. Kidney Int 2007;72:632-637.
15. Stejskal D, Karpisek M, Humenanska V, Hanulova Z, Stejskal P, Kusnierova P, Petzel M. Lipocalin-2: development, analytical characterization, and clinical testing of a new ELISA. Horm Metab Res 2008;40:381-385.
16. Lacher DA, Barletta J, Hughes JP. Biological variation of hematology tests based on the 1999-2002 National Health and Nutrition Examination Survey. Natl Health Stat Report 2012;(54):1-10.
17. Mercadal L, Metzger M, Casadevall N, Haymann JP, Karras A, Boffa JJ, Flamant M, Vrtovsnik F, Stengel B, Froissart M, NephroTest Study Group. Timing and determinants of erythropoietin deficiency in chronic kidney disease. Clin J Am Soc Nephrol 2012;7:35-42.

Experimental differences in pathophysiological pathways between sexes

Our observation, that biomarkers related to inflammation and remodelling were significantly lower in women, might reflect the sex-dependent different aetiology of heart failure, sex-characteristic remodelling pattern, and the influence of comorbidities, all of which are associated with a distinctive increase in biomarkers of inflammation and remodelling. Biologically, the sex differences are most likely attributable to the effects of oestrogen on the corresponding pathophysiological pathways, as shown by experimental data in animals and humans in cardiovascular disease and heart failure.[39]

The main demographic and aetiological sex difference in heart failure is a predominance of myocardial infarctions and the presence of ischaemic heart disease in men over women. Inflammation is one of the key processes in myocardial damage and the post-myocardial infarction remodelling process, which might explain higher inflammatory activation in male heart failure patients. However, there is a well-known profound interaction of female sex and oestrogen with the specific remodelling pattern and the progression to heart failure.[40] Female sex is reliably associated with a slowed and attenuated development of adverse cardiac remodelling and heart failure in various animal models and human studies on myocardial injury,[41,42] pressure[43–46] and volume overload.[47–49] Therefore, it can be speculated if the lower concentrations of inflammatory and remodelling biomarkers in women are to be regarded as a surrogate of less scar or adverse remodelling burden.

Notably, most of the comorbidities, which might potentially confound the levels of inflammation markers by being associated with an increase of respective values, are preferentially seen in women with heart failure. Thus, diabetes,[50] BMI,[51,52] and depression[53] have previously been shown to increase inflammatory biomarkers.

Additionally, age is known to influence the expression of inflammatory markers. With increasing age, the level of expression of inflammatory markers increases in the general population.[54] Notably, with regard to the fact that women were 2.7 years older on average in our study population than men, the

lower level of inflammatory markers among women in our study population of heart failure patients compared with men appears remarkable.

Pathophysiological rationale

There is a strong pathophysiological rationale that the female cardiovascular response to damage is different from that in men. Men are prone to remodelling with LV-dilatation and fibrosis while women more frequently remodel with marked concentric hypertrophy and smaller LV cavity volumes.[9,55] These different mechanistic adaptations imply that heart failure does not necessarily depend on reduction of LVEF, but includes heart failure with preserved EF,[56] which is more common in women.[23] However, arbitrary dichotomization of heart failure into preserved or reduced LVEF, as used in many clinical trials, does not appear to adequately explain sex differences in heart failure presentation and outcome. As Adams et al.[57] demonstrated, female gender is significantly associated with better survival ($P < 0.001$), depending on the primary aetiology of heart failure instead of baseline ventricular function. Women had better survival than men when heart failure aetiology was non-ischaemic. This relationship has also been proven by the results from the BEST study, where the prognostic benefit of non-ischaemic heart failure aetiology was stressed.[58] Our own results in the total COACH cohort match these findings, by showing a pronounced survival benefit for women with non-ischaemic heart disease (31.6 vs. 39.9%, age-adjusted hazard ratio = 0.65; 95% confidence interval 0.45–0.94, $P = 0.022$). While differences in age obviously do not explain the sex difference in survival, a sex difference in symptom and disease burden may. At time of heart failure hospitalization women may present at earlier biological stage of heart failure, while men often present at a pathophysiologically more advanced stage of (mostly ischaemic) cardiomyopathy with already reduced LVEF, translating to a survival disadvantage during the follow-up.

This hypothesis integrates gender (psychosocial) and sex (biological) aspects, and COACH uniquely allows the analysis of both features simultaneously. In our study cohort women did not differ from men regarding NYHA class, and Minnesota Living with Heart failure questionnaire scores at index admission. Although also not significantly differing in terms of current suffering from depression, as defined by a CES-D score ≥16, the rate of depression in women was higher and they showed more concomitant antidepressant use, suggestive for a higher depression prevalence in women. Depression is a common co-morbidity in heart failure, especially in women,[59,60] a finding confirmed in our population. Although it has previously been linked to worse mortality in heart failure with reduced[61] and preserved LVEF,[62] we found no association with all-cause mortality in our cohort, which may be explained by treatment effects related to specific antidepressants or study participation. Although baseline elevations of inflammatory biomarkers such as IL-6 and C-reactive protein have previously been associated with depressive symptoms in the COACH cohort,[63] there was no sex-specific correlation, and in the present sex-specific analysis women had even lower baseline levels of inflammatory markers.

Study limitations

This study is affected by the typical limitations of post hoc analyses, necessitating cautious interpretation. COACH had no specific design to warrant sufficient power for analyses of the sex subgroups. No a priori hypotheses on the sex subgroups were stated in advance. Furthermore, post hoc biomarker analysis in a subset of patients introduces potential selection bias. Assignment of biomarkers to individual pathophysiological process categories is somewhat arbitrary and cannot account for the diverse biological activity of individual markers. The lack of data on oestrogen levels or menopause does not allow respective differentiation. No data regarding previous pregnancies of female patients are available in COACH, which precludes investigation of a link between previous pregnancies and biomarkers. Study inclusion and biomarker sampling in COACH were done just

before discharge, in a stable clinical condition. Therefore, in COACH patients cannot be considered to have acute heart failure, but they are also not completely comparable with chronic heart failure patients. This study is based on biological subgroup classification, is exploratory in nature, and aims to generate new hypotheses. A causal relationship cannot be concluded from the present data and the hypothesis generating results should be confirmed in separate analyses.

Conclusion

Female heart failure patients have a different clinical presentation and better outcomes compared with male patients. Several biomarkers related to inflammation and remodelling were significantly lower in women and NTpro-BNP, GDF-15, TNF-αR1a, MPO, syndecan, galectin 3, and ESAM had sex-dependent prognostic value. Our findings indicate that the biological state of heart failure at admission is less advanced in women compared with men and suggest the sex-specific natural history and course of remodelling may be of particular relevance. There is an unmet need to clarify the pathophysiological processes involved in sex differences in heart failure. Especially in women, current study data are scarce and that requires preferential inclusion of women in clinical trials and related preliminary planning of studies to bridge the gap in current knowledge between men and women.

Ethical approval

The study was approved by the local Ethics Committee and conducted in accordance with Declaration of Helsinki guidelines. All patients provided written informed consent. Additional consent was obtained for 36-month follow-up.

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References

1. Mehta PA, Cowie MR. Gender and heart failure: a population perspective. *Hear 92 Suppl i*. 2006;3:ii14–8.
2. Lindenfeld J, Krause-Steinrauf H, Salerno J. Where are all the women with heart failure? *J Am Coll Cardiol*. 1997;30:1417–1419.
3. Heiat A, Gross CP, Krumholz HM. Representation of the elderly, women, and minorities in heart failure clinical trials. *Arch Intern Med*. 2002;162:1682–1688.
4. Hsich EM, Piña IL. Heart failure in women: a need for prospective data. *J Am Coll Cardiol*. 2009;54:491–498.
5. Rathore SS, Wang Y, Krumholz HM. Sex-based differences in the effect of digoxin for the treatment of heart failure. *N Engl J Med*. 2002;347:1403–1411.
6. O'Meara E, Clayton T, McEntegart MB, McMurray JJ V, Piña IL, Granger CB, Ostergren J, Michelson EL, Solomon SD, Pocock S, Yusuf S, Swedberg K, Pfeffer MA. Sex differences in clinical characteristics and prognosis in a broad spectrum of patients with heart failure: results of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) program. *Circulation*. 2007;115:3111–20.
7. Simon T, Mary-Krause M, Funck-Brentano C, Jaillon P. Sex differences in the prognosis of congestive heart failure: results from the Cardiac Insufficiency Bisoprolol Study (CIBIS II). *Circulation*. 2001;103:375–380.
8. Arshad A, Moss AJ, Foster E, Padeletti L, Barsheshet A, Goldenberg I, Greenberg H, Hall WJ, McNitt S, Zareba W, Solomon S, Steinberg JS, MADIT-CRT Executive Committee. Cardiac resynchronization therapy is more effective in women than in men: the MADIT-CRT (Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy) trial. *J Am Coll Cardiol*. 2011;57:813–820.
9. Piro M, Della Bona R, Abbate A, Biasucci LM, Crea F. Sex-related differences in myocardial

remodeling. *J Am Coll Cardiol*. 2010;55:1057–1065.

10. Jaarsma T, Van Der Wal MHL, Hogenhuis J, Lesman I, Luttik M-LA, Veeger NJGM, Van Veldhuisen DJ. Design and methodology of the COACH study: a multicenter randomised Coordinating study evaluating Outcomes of Advising and Counselling in Heart failure. *Eur J Heart Fail*. 2004;6:227–233.
11. Jaarsma T, van der Wal MHL, Lesman-Leegte I, Luttik M-L, Hogenhuis J, Veeger NJ, Sanderman R, Hoes AW, van Gilst WH, Lok DJA, Dunselman PHJM, Tijssen JGP, Hillege HL, van Veldhuisen DJ, of Advising CSEO, in Heart Failure (COACH) Investigators. Effect of moderate or intensive disease management program on outcome in patients with heart failure: Coordinating Study Evaluating Outcomes of Advising and Counseling in Heart Failure (COACH). *Arch Intern Med*. 2008;168:316–324.
12. Liu LCY, Voors AA, van Veldhuisen DJ, van der Veer E, Belonje AM, Szymanski MK, Silljé HHW, van Gilst WH, Jaarsma T, de Boer RA. Vitamin D status and outcomes in heart failure patients. *Eur J Heart Fail*. 2011;13:619–625.
13. Linssen GCM, Rienstra M, Jaarsma T, Voors AA, van Gelder IC, Hillege HL, van Veldhuisen DJ. Clinical and prognostic effects of atrial fibrillation in heart failure patients with reduced and preserved left ventricular ejection fraction. *Eur J Heart Fail*. 2011;13:1111–1120.
14. de Boer RA, Lok DJA, Jaarsma T, van der Meer P, Voors AA, Hillege HL, van Veldhuisen DJ. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med*. 2011;43:60–8.
15. Radloff LS, The CES. D scale. *A selfreport Depress scale Res Gen Popul Psychol Meas Appl*. 1977;1:385–401.
16. Schroevers MJ, Sanderman R, van Sonderen E, Ranchor A V. The evaluation of the Center for Epidemiologic Studies Depression (CES-D) scale: Depressed and Positive Affect in cancer patients and healthy reference subjects. *Qual Life Res*. 2000;9:1015–1029.

17. Rector TS, Kubo SH, Cohn JN. Patients' self-assessment of their congestive heart failure: content, reliability and validity of a new measure, the Minnesota Living with Heart Failure questionnaire. *Hear Fail*. 1987;3:198–209.

18. Postmus D, van Veldhuisen DJ, Jaarsma T, Luttik ML, Lassus J, Mebazaa A, Nieminen MS, Harjola V-P, Lewsey J, Buskens E, Hillege HL. The COACH risk engine: a multistate model for predicting survival and hospitalization in patients with heart failure. *Eur J Heart Fail*. 2012;14:168–175.

19. Vaccarino V, Chen YT, Wang Y, Radford MJ, Krumholz HM. Sex differences in the clinical care and outcomes of congestive heart failure in the elderly. *Am Heart J*. 1999;138:835–842.

20. Galvao M, Kalman J, DeMarco T, Fonarow GC, Galvin C, Ghali JK, Moskowitz RM. Gender differences in in-hospital management and outcomes in patients with decompensated heart failure: analysis from the Acute Decompensated Heart Failure National Registry (ADHERE). *J Card Fail*. 2006;12:100–107.

21. Nieminen MS, Harjola V-P, Hochadel M, Drexler H, Komajda M, Brutsaert D, Dickstein K, Ponikowski P, Tavazzi L, Follath F, Lopez-Sendon JL. Gender related differences in patients presenting with acute heart failure. Results from EuroHeart Failure Survey II. *Eur J Heart Fail*. 2008;10:140–148.

22. Alla F, Al-Hindi AY, Lee CR, Schwartz TA, Patterson JH, Adams KF. Relation of sex to morbidity and mortality in patients with heart failure and reduced or preserved left ventricular ejection fraction. *Am Heart J*. 2007;153:1074–1080.

23. Ho JE, Gona P, Pencina MJ, Tu J V, Austin PC, Vasan RS, Kannel WB, D'Agostino RB, Lee DS, Levy D. Discriminating clinical features of heart failure with preserved vs. reduced ejection fraction in the community. *Eur Heart J*. 2012;33:1734–1741.

24. Tasevska-Dinevska G, Kennedy LMA, Nilsson PM, Willenheimer R. Gender aspects on heart failure incidence and mortality in a middle-aged, urban, community-based population sample:

the Malmö preventive project. *Eur J Epidemiol*. 2009;24:249–257.

25. Martínez-Sellés M, Doughty RN, Poppe K, Whalley GA, Earle N, Tribouilloy C, McMurray JJ V, Swedberg K, Køber L, Berry C, Squire I. Gender and survival in patients with heart failure: interactions with diabetes and aetiology. Results from the MAGGIC individual patient meta-analysis. *Eur J Heart Fail*. 2012;14:473–9.
26. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB, Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. *Am Heart J*. 2006;152:593–8.
27. Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K. Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. *Clin Chem Lab Med*. 2009;47:471–477.
28. Norata GD, Garlaschelli K, Grigore L, Tibolla G, Raselli S, Redaelli L, Buccianti G, Catapano AL. Circulating soluble receptor for advanced glycation end products is inversely associated with body mass index and waist/hip ratio in the general population. *Nutr Metab Cardiovasc Dis*. 2009;19:129–34.
29. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, Bakker SJL, van der Harst P. The fibrosis marker galectin-3 and outcome in the general population. *J Intern Med*. 2012;272:55–64.
30. Wang TJ, Larson MG, Levy D, Leip EP, Benjamin EJ, Wilson PWF, Sutherland P, Omland T, Vasan RS. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. *Am J Cardiol*. 2002;90:254–258.
31. Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol*. 2007;50:1054–60.
32. Stejskal D, Karpísek M, Humenanska V, Hanulova Z, Stejskal P, Kusnierova P, Petzel M.

Lipocalin-2: development, analytical characterization, and clinical testing of a new ELISA. *Horm Metab Res = Horm und Stoffwechselforsch = Horm métabolisme*. 2008;40:381–5.

33. Mercadal L, Metzger M, Casadevall N, Haymann JP, Karras A, Boffa J-J, Flamant M, Vrtovsnik F, Stengel B, Froissart M. Timing and determinants of erythropoietin deficiency in chronic kidney disease. *Clin J Am Soc Nephrol*. 2012;7:35–42.
34. Marques-Vidal P, Bochud M, Bastardot F, Lüscher T, Ferrero F, Gaspoz J-M, Paccaud F, Urwyler A, von Känel R, Hock C, Waeber G, Preisig M, Vollenweider P. Levels and determinants of inflammatory biomarkers in a Swiss population-based sample (CoLaus study). *PLoS One*. 2011;6:e21002.
35. Lin Y, Nakachi K, Ito Y, Kikuchi S, Tamakoshi A, Yagyu K, Watanabe Y, Inaba Y, Kazuo Tajima. Variations in serum transforming growth factor-beta1 levels with gender, age and lifestyle factors of healthy Japanese adults. *Dis Markers*. 2009;27:23–8.
36. Rohatgi A, Patel P, Das SR, Ayers CR, Khera A, Martinez-Rumayor A, Berry JD, McGuire DK, de Lemos JA. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. *Clin Chem*. 2012;58:172–182.
37. Wetzels JFM, Kiemeney LALM, Swinkels DW, Willems HL, den Heijer M. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. *Kidney Int*. 2007;72:632–7.
38. Lacher DA, Barletta J, Hughes JP. Biological variation of hematology tests based on the 1999-2002 National Health and Nutrition Examination Survey. *Natl Health Stat Report*. 2012;1–10.
39. Du X-J, Fang L, Kiriazis H. Sex dimorphism in cardiac pathophysiology: experimental findings, hormonal mechanisms, and molecular mechanisms. *Pharmacol Ther*. 2006;111:434–475.
40. Fliegner D, Schubert C, Penkalla A, Witt H, Kararigas G, Kararigas G, Dworatzek E, Staub E, Martus P, Ruiz Noppinger P, Kintscher U, Gustafsson J-A, Regitz-Zagrosek V. Female sex

and estrogen receptor-beta attenuate cardiac remodeling and apoptosis in pressure overload. *Am J Physiol Regul Integr Comp Physiol*. 2010;298:R1597–606.

41. Cavasin MA, Tao Z, Menon S, Yang X-P. Gender differences in cardiac function during early remodeling after acute myocardial infarction in mice. *Life Sci*. 2004;75:2181–92.
42. Wu JC, Nasseri BA, Bloch KD, Picard MH, Scherrer-Crosbie M. Influence of sex on ventricular remodeling after myocardial infarction in mice. *J Am Soc Echocardiogr*. 2003;16:1158–1162.
43. Pfeffer JM, Pfeffer MA, Fletcher P, Fishbein MC, Braunwald E. Favorable effects of therapy on cardiac performance in spontaneously hypertensive rats. *Am J Physiol*. 1982;242:H776–84.
44. Jain M, Liao R, Podesser BK, Ngoy S, Apstein CS, Eberli FR. Influence of gender on the response to hemodynamic overload after myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2002;283:H2544–50.
45. Douglas PS, Katz SE, Weinberg EO, Chen MH, Bishop SP, Lorell BH. Hypertrophic remodeling: gender differences in the early response to left ventricular pressure overload. *J Am Coll Cardiol*. 1998;32:1118–1125.
46. Rohde LE, Zhi G, Aranki SF, Beckel NE, Lee RT, Reimold SC. Gender-associated differences in left ventricular geometry in patients with aortic valve disease and effect of distinct overload subsets. *Am J Cardiol*. 1997;80:475–480.
47. Gardner JD, Brower GL, Janicki JS. Gender differences in cardiac remodeling secondary to chronic volume overload. *J Card Fail*. 2002;8:101–107.
48. Brower GL, Gardner JD, Janicki JS. Gender mediated cardiac protection from adverse ventricular remodeling is abolished by ovariectomy. *Mol Cell Biochem*. 2003;251:89–95.
49. Dent MR, Tappia PS, Dhalla NS. Gender differences in cardiac dysfunction and remodeling due to volume overload. *J Card Fail*. 2010;16:439–449.
50. Saltevo J, Kautiainen H, Vanhala M. Gender differences in adiponectin and low-grade

inflammation among individuals with normal glucose tolerance, prediabetes, and type 2 diabetes. *Gend Med*. 2009;6:463–470.

51. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. *Diabetes Care*. 1999;22:1971–1977.

52. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA*. 1999;282:2131–2135.

53. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med*. 2009;71:171–186.

54. Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, Guralnik JM, Longo DL. The origins of age-related proinflammatory state. *Blood*. 2005;105:2294–2299.

55. Du XJ, Fang L, Kiriazis H. Sex dimorphism in cardiac pathophysiology: Experimental findings, hormonal mechanisms, and molecular mechanisms. *Pharmacol Ther*. 2006;111:434–475.

56. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. *Eur Heart J*. 2011;32:670–679.

57. Adams KF, Dunlap SH, Sueta CA, Clarke SW, Patterson JH, Blauwet MB, Jensen LR, Tomasko L, Koch G. Relation between gender, etiology and survival in patients with symptomatic heart failure. *J Am Coll Cardiol*. 1996;28:1781–1788.

58. Ghali JK, Krause-Steinrauf HJ, Adams KF, Khan SS, Rosenberg YD, Yancy CW, Young JB, Goldman S, Peberdy MA, Lindenfeld J. Gender differences in advanced heart failure: insights from the BEST study. *J Am Coll Cardiol*. 2003;42:2128–2134.

59. Gottlieb SS, Khatta M, Friedmann E, Einbinder L, Katzen S, Baker B, Marshall J, Minshall S, Robinson S, Fisher ML, Potenza M, Sigler B, Baldwin C, Thomas SA. The influence of age, gender, and race on the prevalence of depression in heart failure patients. *J Am Coll Cardiol*. 2004;43:1542–1549.

60. Rutledge T, Reis VA, Linke SE, Greenberg BH, Mills PJ. Depression in heart failure a meta-

analytic review of prevalence, intervention effects, and associations with clinical outcomes. *J Am Coll Cardiol*. 2006;48:1527–1537.

61. Jiang W, Kuchibhatla M, Clary GL, Cuffe MS, Christopher EJ, Alexander JD, Califf RM, Krishnan RR, O'Connor CM. Relationship between depressive symptoms and long-term mortality in patients with heart failure. *Am Heart J*. 2007;154:102–108.

62. Kato N, Kinugawa K, Shiga T, Hatano M, Takeda N, Imai Y, Watanabe M, Yao A, Hirata Y, Kazuma K, Nagai R. Depressive symptoms are common and associated with adverse clinical outcomes in heart failure with reduced and preserved ejection fraction. *J Cardiol*. 2012;60:23–30.

63. Johansson P, Lesman-Leegte I, Svensson E, Voors A, van Veldhuisen DJ, Jaarsma T. Depressive symptoms and inflammation in patients hospitalized for heart failure. *Am Heart J*. 2011;161:1053–1059.